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April 21, 2005

Examiner Q. Janice Li
U.S. Patent & Trademark Office
Commissioner for Patents
Washington, D.C. 20231

Re: LIVE VACCINES FOR ALLERGY TREATMENT
Appli. No.: 09/778,672
Our Ref.: 12774-002001

Dear Examiner Li:

Thank you for conducting a telephone interview on March 8, 2005 ("the previous interview") and granting a second telephone interview, scheduled for 4:00 pm, April 21, 2005 to resolve an issue raised in the final office action and advisory action.

During the previous interview, we discussed independent claim 24. This claim is drawn to a method of decreasing IgE production in a subject exposed to a dust mite allergen. The method includes orally administering to a subject a non-pathogenic Gram-positive bacterium that contains a nucleotide sequence encoding a dust mite allergen; and expressing the allergen in the bacterium.

You rejected this claim for obviousness over **Hsu** (which teaches suppressing allergen-specific IgE production by administering to a subject a plasmid encoding an allergen) and **Janeway** (which teaches shifting an antibody response away from an IgE-dominated response towards one dominated by IgG for desensitization) in view of **Pouwels** and **Medaglini** (both of which teach using an antigen/allergen expressed by non-pathogenic Gram-positive bacteria for oral immunization). You asserted that it would have been obvious to one skilled person to modify Hsu's method by replacing the

daglini.

During the previous interview, neither motivation nor reasonable exp manner you proposed. More specifically, v Cell by Bruce Alberts et al., Garland Pub; 4th aches that an antigen delivered by a bacteriu ed by CD4+ T cells. Hsu teaches that "CD4+)." In view of the knowledge, a skilled artisa in Pouwels/Medaglini to suppres

please scan, not mail
thanks

For your convenience, we hav sent to you before the previous interview. hand.

Very truly yours,

Y. Rocky Tsao

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EXHIBIT

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March 7, 2005

Examiner Q. Janice Li
U.S. Patent & Trademark Office
Commissioner for Patents
Washington, D.C. 20231

Re: LIVE VACCINES FOR ALLERGY TREATMENT
Appli. No.: 09/778,672
Our Ref.: 12774-002001

FR

AUSTIN
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SAN DIEGO
SILICON VALLEY
TWIN CITIES
WASHINGTON, DC

Dear Examiner Li:

Thank you for granting a telephone interview, scheduled for 2:30 pm, March 8, 2005 to resolve an issue raised in the final office action and advisory action. As I know you are busy, I will limit the telephone discussion to claim 24. Hopefully, the summary below will facilitate resolving this issue.

Claim 24, rejected for obviousness, is drawn to a method of decreasing the production of IgE in a subject exposed to a dust mite allergen. The method includes orally administering to a subject a non-pathogenic Gram-positive bacterium that contains a nucleotide sequence encoding a dust mite allergen; and expressing the allergen in the bacterium.

You rejected this claim as being obvious over Hsu (which teaches suppressing allergen-specific IgE production in a subject by administering to a subject a recombinant plasmid encoding an allergen) and Janeway (which teaches shifting an antibody response away from an IgE-dominated response towards one dominated by IgG for desensitization) in view of Pouwels and Medaglini (both of which teach using an antigen or allergen expressed by non-pathogenic Gram-positive bacteria for oral immunization). You asserted that it would have been obvious to one skilled person to modify the Hsu's method by replacing the recombinant plasmid with the bacterium taught in Pouwels or Medaglini.

In the response to the final office action, we pointed to a paragraph from Hsu to show that (1) the Hsu's plasmid-based approach relies on the CD8+ T cell-dependent antigen-presenting pathway and suppresses IgE production; and (2) the Pouwels or Medaglini bacterium-based approach relies on the $CD4^+$ T cell-dependent antigen-presenting pathway and supports IgE production. We proceeded to conclude that a skilled person would not use the bacteria taught in Pouwels or Medaglini for suppressing IgE.

You countered that the Hsu paragraph regarding the two pathways is not specific to the above-mentioned two approaches. To elucidate these two pathways, we are attaching three relevant figures from Molecular Biology of the Cell by Bruce Alberts et al., Garland Pub; 4th edition, March 2002 ("Alberts"). (The table appended to this letter shows the features of the two pathways, as well as their implication in IgE regulation as taught in Hsu.)

Figure 24-58 illustrates the antigen-presentation of a foreign protein (e.g., a viral protein) that is generated inside an antigen-presenting cell (APC) of a subject. This "self-made" foreign protein is bound to class I MHC protein and recognized by CD8+ T cells (also see Figure 24-55). Note that Figure 24-58 mirrors the Hsu approach. More specifically, a recombinant plasmid encoding a foreign allergen gene is introduced into an APC. This foreign gene is then transcribed and translated inside the cell, processed through cytoplasm and proreasome, bound to class I MHC protein, and recognized by CD8+ T cells. In contrast, Figure 24-60 represents the Pouwels or Medaglini approach, in which an antigen or allergen is already expressed by bacteria as an extracellular protein to an APC. This extracellular protein is then endocytosed by the cell, processed without going through cytoplasm or proreasome, bound to class II MHC protein, and finally recognized by CD4+ T cells.

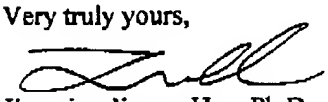
In view the above knowledge well known in the art and Hsu's teachings on IgE regulation, a skilled person would recognize that an antigen delivered by the Pouwels or Medaglini bacterium is recognized by CD4+ T cells, which support IgE production. It follows that he or she would not have been motivated to replace the Hsu's recombinant plasmid with the bacteria taught in Pouwels or Medaglini.

Janeway teaches desensitizing by shifting an antibody response away from an IgE-dominated response towards an IgG dominated response. For the same reasons set forth above, a skilled person would have recognized that Janeway's teaching is applicable to the Hsu plasmid-based approach only, but not to the Pouwels or Medaglini bacterium-based approach. In other words, a skilled person would have no reasonable expectation of success to modify Hsu's method in the manner you suggested.

Here, we note that "[t]he test for obviousness is what the combined teachings of the references would have suggested to one of ordinary skill in the art, and all teachings in the prior art must be considered to the extent that they are in analogous arts." See MPEP 2143.01. Thus, all above-discussed references must be considered. It is improper to consider only Janeway and disregard Hsu or Alberts.

We look forward to discussing with you the above issue at the interview.

Very truly yours,



Jianming Jimmy Hao, Ph.D.
Reg. No. 54,694

Table. Comparison of Two Antigen-presenting Pathways

Figure	Antigen	Cell organelles	Bound to	Recognized by	Examples	IgE
Figure 24-58	A foreign gene is transcribed and translated inside an APC.	Goes through cytoplasm and proteasome	Class I MHC	CD8+ T cells.	Hsu	Suppress IgE production
Figure 24-60	A foreign gene is already expressed as an extracellular protein to an APC.	Does not go through cytoplasm or proteasome	Class II MHC	CD4+ T cells	Pouwels Medaglini	Support IgE production

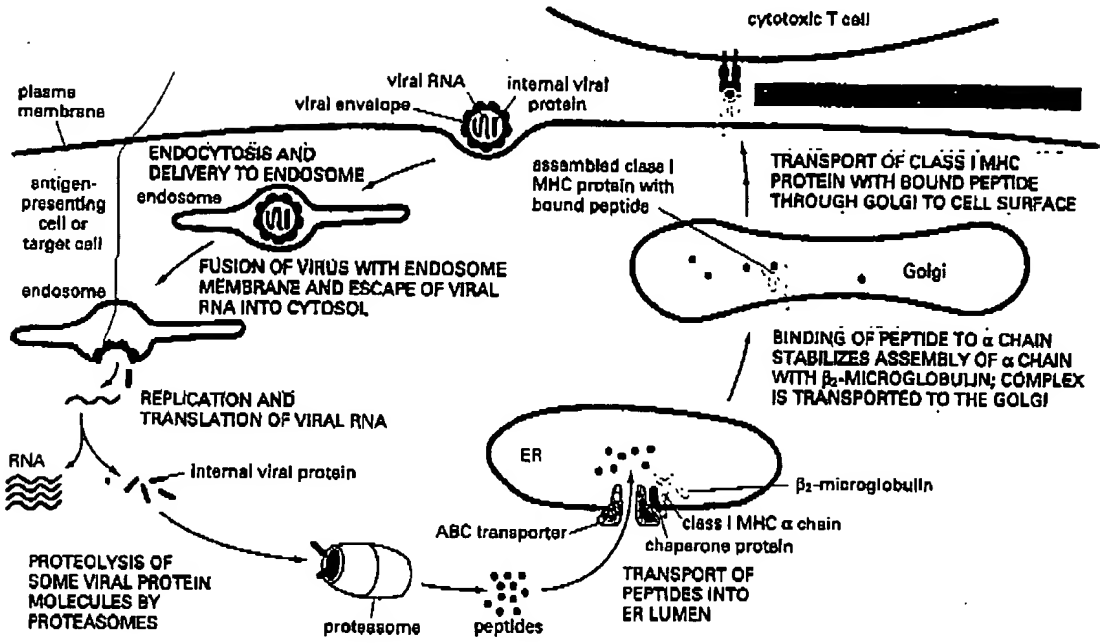


Figure 24–58 The processing of a viral protein for presentation to cytotoxic T cells. An effector cytotoxic T cell kills a virus-infected cell when it recognizes fragments of viral protein bound to class I MHC proteins on the surface of the infected cell. Not all viruses enter the cell in the way that this enveloped RNA virus does, but fragments of internal viral proteins always follow the pathway shown. Some of the viral proteins synthesized in the cytosol are degraded, and this is a sufficient amount to attract an attack by a cytotoxic T cell. The folding and assembly of a class I MHC protein is aided by several chaperone proteins in the ER lumen, only one of which is shown. The chaperones bind to the class I MHCα chain and act sequentially. The last one binds the MHC protein to the ABC transporter, as shown.

In cells that are not infected, peptide fragments come from the cells' own cytosolic and nuclear proteins that are degraded in the processes of normal protein turnover and quality control mechanisms. (Surprisingly, more than 30% of the proteins made by mammalian cells are apparently faulty and are degraded in proteasomes soon after they are synthesized.) These peptides are pumped into the ER and are carried to the cell surface by class I MHC proteins. They are not antigenic because the cytotoxic T cells that could recognize them have been eliminated or inactivated during T cell development, as we discuss later.

When cytotoxic T cells and some helper T cells are activated by antigen to become effector cells, they secrete the cytokine **interferon-γ (IFN-γ)**, which greatly enhances anti-viral responses. The IFN-γ acts on infected cells in two ways. It blocks viral replication, and it increases the expression of many genes within the MHC chromosomal region. These genes include those that encode class I (and class II) MHC proteins, the two specialized proteasome subunits, and the two subunits of the peptide transporter located in the ER (Figure 24–59). Thus, all of the machinery required for presenting viral antigens to cytotoxic T cells is coordinately called into action by IFN-γ, creating a positive feedback that amplifies the immune response and culminates in the death of the infected cells.

Helper T Cells Recognize Fragments of Endocytosed Foreign Protein Associated with Class II MHC Proteins

Unlike cytotoxic T cells, helper T cells do not act directly to kill infected cells so as to eliminate microbes. Instead, they stimulate macrophages to be more effective in destroying intracellular microorganisms, and they help B cells and cytotoxic T cells to respond to microbial antigens.

Like the viral proteins presented to cytotoxic T cells, the proteins presented to helper T cells on antigen-presenting cells or target cells are degraded fragments of foreign proteins. The fragments are bound to class II MHC proteins in much the same way that virus-derived peptides are bound to class I MHC proteins. But both the source of the peptide fragments presented and the route they take to find the MHC proteins are different from those of peptide fragments presented by class I MHC proteins to cytotoxic T cells.

Rather than being derived from foreign protein synthesized in the cytosol of a cell, the foreign peptides presented to helper T cells are derived from endosomes. Some come from extracellular microbes or their products that the

mice that express a specific pair of rearranged α and β T cell receptor genes derived from a T cell clone of known antigen and MHC specificity. Such experiments show that the transgenic T cells mature in the thymus and populate the peripheral lymphoid organs only if the transgenic mouse also expresses the same allelic form of MHC protein as is recognized by the transgenic T cell receptor. If the mouse does not express the appropriate MHC protein, the transgenic T cells die in the thymus. Thus, the survival and maturation of a T cell depend on a match between its receptor and the MHC proteins expressed in the thymus. Similar experiments using transgenic mice in which MHC expression is confined to specific cell types in the thymus indicate that it is MHC proteins on epithelial cells in the cortex of the thymus that are responsible for this positive selection process. After positively selected T cells leave the thymus, their continued survival depends on their continual stimulation by self-peptide–MHC complexes; this stimulation is enough to promote cell survival but not enough to activate the T cells to become effector cells.

As part of the positive selection process in the thymus, developing T cells that express receptors recognizing class I MHC proteins are selected to become cytotoxic cells, while T cells that express receptors recognizing class II MHC proteins are selected to become helper cells. Thus, genetically engineered mice that lack cell-surface class I MHC proteins specifically lack cytotoxic T cells, whereas mice that lack class II MHC proteins specifically lack helper T cells. The cells that are undergoing positive selection initially express both CD4 and CD8 co-receptors, and these are required for the selection process: without CD4, helper T cells fail to develop, and without CD8, cytotoxic T cells fail to develop.

Positive selection still leaves a large problem to be solved. If developing T cells with receptors that recognize self peptides associated with self MHC proteins were to mature in the thymus and migrate to peripheral lymphoid tissues, they might wreak havoc. A second, *negative selection* process in the thymus is required to help avoid this potential disaster.

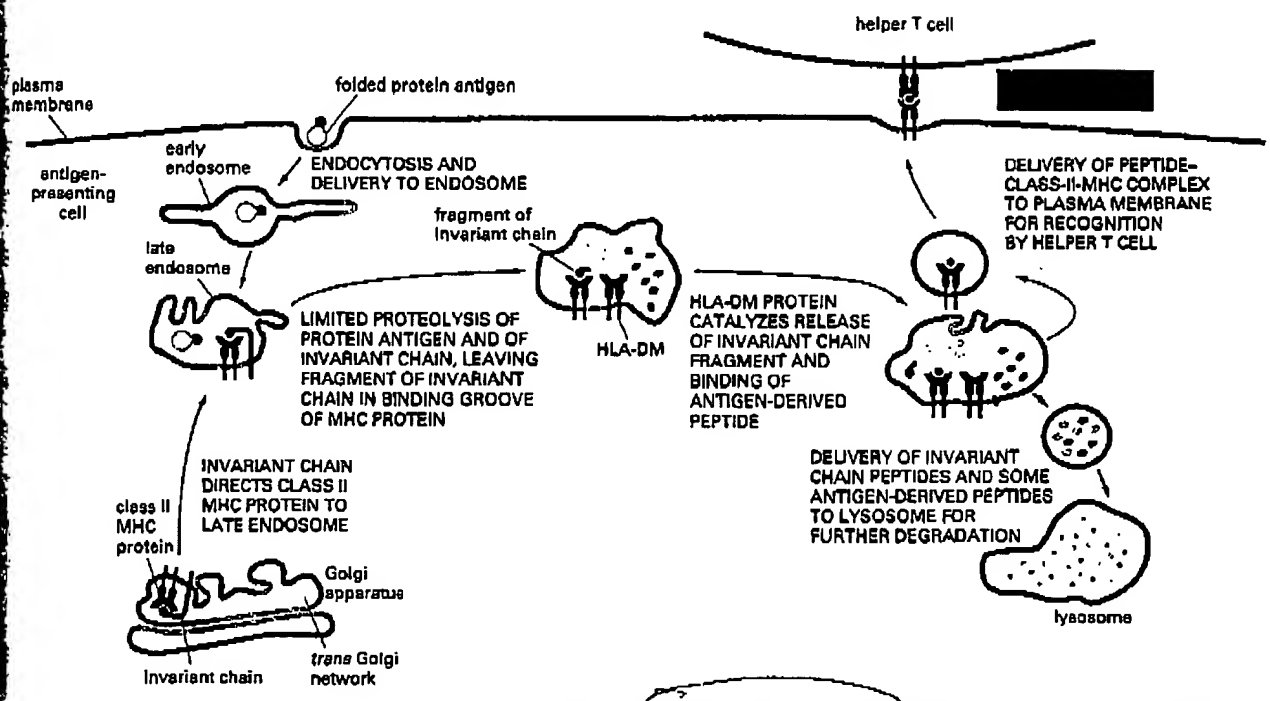


Figure 24–60 The processing of an extracellular protein antigen for presentation to a helper T cell. The drawing shows a simplified view of how peptide–class-II-MHC complexes are formed in endosomes and delivered to the cell surface. Note that the release of the invariant-chain fragment from the binding groove of the class II MHC protein in the endosome is catalyzed by a class-II-MHC-like protein called HLA-DM. Viral glycoproteins can also be processed by this pathway for presentation to helper T cells. They are made in the ER, are transported to the plasma membrane, and can then enter endosomes after endocytosis.

TABLE 24–2 Properties of Human Class I and Class II MHC Proteins

	CLASS I	CLASS II
Genetic loci	*HLA-A, HLA-B, HLA-C*	*DP, DQ, DR*
Chain structure	α chain + β_2-microglobulin	α chain + β chain
Cell distribution	most nucleated cells	antigen-presenting cells (including B cells), thymus epithelial cells, some others
Involved in presenting antigen to	cytotoxic T cells	helper T cells
Source of peptide fragments	proteins made in cytoplasm	endocytosed plasma membrane and extracellular proteins
Polymorphic domains	$\alpha_1 + \alpha_2$	$\alpha_1 + \beta_1$
Recognition by co-receptor	CD8	CD4

CD4 and CD8 Co-receptors Bind to Nonvariable Parts of MHC Proteins

The affinity of T cell receptors for peptide–MHC complexes on an antigen-presenting cell or target cell is usually too low to mediate a functional interaction between the two cells by itself. T cells normally require *accessory receptors* to help stabilize the interaction by increasing the overall strength of the cell–cell adhesion. Unlike T cell receptors or MHC proteins, the accessory receptors do not bind foreign antigens and are invariant.

When accessory receptors also have a direct role in activating the T cell by generating their own intracellular signals, they are called **co-receptors**. The most important and best understood of the co-receptors on T cells are the CD4 and CD8 proteins, both of which are single-pass transmembrane proteins with extracellular Ig-like domains. Like T cell receptors, they recognize MHC proteins, but, unlike T cell receptors, they bind to nonvariable parts of the protein, far away from the peptide-binding groove. **CD4** is expressed on helper T cells and binds to class II MHC proteins, whereas **CD8** is expressed on cytotoxic T cells and binds to class I MHC proteins (Figure 24–55). Thus, CD4 and CD8 contribute to T cell recognition by helping to focus the cell on particular MHC proteins, and thus on particular types of cells—helper T cells on dendritic cells, macrophages, and B cells, and cytotoxic cells on any nucleated host cell displaying a foreign peptide on a class I MHC protein. The cytoplasmic tail of these transmembrane proteins is associated with a member of the Src family of cytoplasmic tyrosine protein kinases called *Lck*, which phosphorylates various intracellular proteins on tyrosines and thereby participates in the activation of the T cell. Antibodies to CD4 and CD8 are widely used as tools to distinguish between the two main classes of T cells, in both humans and experimental animals.

Ironically, the AIDS virus (HIV) makes use of CD4 molecules (as well as chemokine receptors) to enter helper T cells. It is the eventual depletion of helper T cells that renders AIDS patients susceptible to infection by microbes that are not normally dangerous. As a result, most AIDS patients die of infection within several years of the onset of symptoms, unless they are treated with a combination of powerful anti-HIV drugs. HIV also uses CD4 and chemokine receptors to enter macrophages, which also have both of these receptors on their surface.

Before a cytotoxic or helper T cell can recognize a foreign protein, the protein has to be processed inside an antigen-presenting cell or target cell so that it can be displayed as peptide–MHC complexes on the cell surface. We first consider how a virus-infected antigen-presenting cell or target cell processes viral proteins for presentation to a cytotoxic T cell. We then discuss how ingested foreign proteins are processed for presentation to a helper T cell.

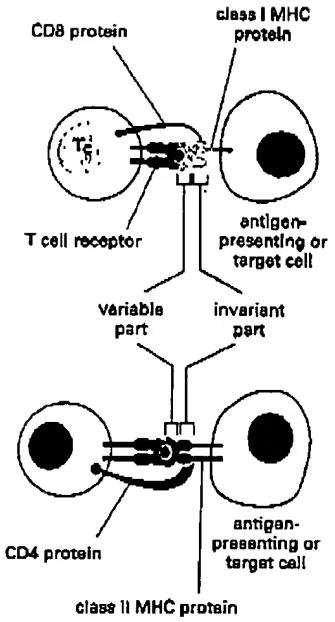


Figure 24–55 CD4 and CD8 co-receptors on the surface of T cells. Cytotoxic T cells (T_C) express CD8, which recognizes class I MHC proteins, whereas helper T cells (T_H) express CD4, which recognizes class II MHC proteins. Note that the co-receptors bind to the same MHC protein that the T cell receptor has engaged, so that they are brought together with T cell receptors during the antigen recognition process. Whereas the T cell receptor binds to the variable (polymorphic) parts of the MHC protein that form the peptide-binding groove, the co-receptor binds to the invariant part, far away from the groove.